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The regulation of GLUT5 and GLUT2 activity in the adaptation of intestinal brush-border fructose transport in diabetes

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Abstract The adaptation of D-fructose transport in rat jejunum to experimental diabetes has been studied. In vivo and in vitro perfusions of intact jejunum with D-fructose revealed the appearance of a phloretin-sensitive transporter in the brush-border membrane of streptozotocin-diabetic rats which was not detectable in normal rats. The nature of the transporters involved was investigated by Western blotting and by D-fructose transport studies using highly purified brush-border and basolateral membrane vesicles. GLUT5, the major transporter in the brush-border membrane of normal rats, was not inhibited by D-glucose or phloretin. In contrast, GLUT2, the major transporter in the basolateral membrane of normal rats, was strongly inhibited by both D-glucose and phloretin. In brush-border membrane vesicles from diabetic rats, GLUT5 levels were significantly enhanced; moreover the presence of GLUT2 was readily detectable and increased markedly as diabetes progressed. The differences in stereospecificity between GLUT2 and GLUT5 were used to show that both transporters contributed to the overall enhancement of D-fructose transport measured in brush-border membrane vesicles and in vitro isolated loops from diabetic rats. However, overall D-fructose uptake in vivo was diminished. The underlying mechanisms and functional consequences are discussed.

Key words Intestine · Transport · Fructose · GLUT5 · GLUT2 · Diabetes

Introduction

Glucose transport across the small intestine of normal rats occurs via the active Na^+/D-glucose cotransporter, SGLT1, of the brush-border membrane [1, 2] and the facilitative transporter, GLUT2, of the basolateral membrane [3]. Diabetes results in enhanced absorption of glucose across both brush-border and basolateral membranes in the mid-villus region [4, 5]. Enhancement involves a significant increase in the mid-villus density of SGLT1 in the brush-border membrane, as determined by [^{3}H]phlorizin binding [4], and also of GLUT2 in the basolateral membrane, as determined by Western blotting [6]. The increases in SGLT1 and GLUT2 densities are associated with increases in their respective mRNAs [7].

Studies using human small intestine have shown that another member of the facilitative glucose transporter family, GLUT5, is present in the brush-border membrane [8]. When expressed in oocytes, human GLUT5 behaves as a high-affinity D-fructose transporter with a much lower capacity to transport D-glucose and its analogues [9]. The brush-border membrane of rat also contains a distinct transport system, which is highly specific for D-fructose and is not inhibited by D-glucose even at ratios as high as 100:1 [10, 11]. The transporter is presumably GLUT5, although it has not been formally identified as such. Although GLUT5 is present in the basolateral membrane of normal human jejunum [12], it is thought not to be present in the basolateral membrane of normal rats raised on a standard chow diet. Instead, D-fructose is transported across the basolateral membrane by GLUT2 [3], as suggested by its ability to transport D-fructose efficiently when expressed in oocytes [13] and demonstrated by the mutual and complete inhibition of D-glucose and D-fructose transport in rat basolateral membrane vesicles [14]. Rat mucosal GLUT5 mRNA is diminished in experimental diabetes [7] and, as noted, a significant increase also occurs in the density of GLUT2 in the basolateral membrane, which parallels an increase in GLUT2 mRNA.

Fructose has been recommended as a substitute for glucose and sucrose in the diets of diabetic and obese people, being sweeter, more soluble and less glucogenic than either sugar. However, in contrast to glucose, studies of the adaptation of intestinal fructose transport to diabetes have been largely neglected. We have therefore

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investigated how fructose transport across the brush-border of rat jejunum adapts to experimental diabetes. A preliminary report of part of this work has been presented as a conference proceeding [15].

Materials and methods

Animals

Male Wistar rats (240–250 g) were fed ad libitum on standard Bantin and Kingman rat and mouse diet with free access to water. Rats were made diabetic by injection, through the tail vein, of streptozotocin (65 mg/kg) freshly made in 0.3 ml of 3.5 mM citric acid, 6.5 mM trisodium citrate buffer, pH 5.0; only those rats having a blood glucose concentration in excess of 25 mM (Bochringer BM-test 1-44 strip) were used in subsequent experiments.

Perfusion of jejunal loops

Before operation, rats were anaesthetised by an intraperitoneal injection of sodium pentabarbitone, 110 mg per kg body weight (Sagatal, Rhone Merieux, Harlow, Essex, UK; 0.14 ml per 100 g body weight). The luminal perfusion of isolated jejunal loops in vivo using a gas-segmented, recirculated flow system has been described in detail previously [16]. This system was modified to a single-pass perfusion system that allowed switching between two luminal perfusate reservoirs (110 ml) to permit a paired comparison of a control and an experimental perfusion period for a single loop. The first reservoir contained 5 mM D-fructose and was used for the control period of 0–60 min, whereas the second contained 5 mM D-fructose and 0.5 mM phloretin and was used for the experimental period from 60 min to 120 min: both reservoirs contained 0.5 mM hydroxybutyrate as an energy substrate. Additional perfusions were performed in which phloretin was not present during the experimental period; these showed that there was no falling off in the D-fructose uptake rate, confirming that the preparation was viable for the whole perfusion period. The luminal perfusion of isolated jejunal loops in vitro using a gas-segmented, recirculated flow system has also been described in detail previously [17]. The system was again modified to include two reservoirs (40 ml each) for control and experimental periods, which contained the same solutions as used for in vivo perfusions. The rate of D-fructose uptake was determined by its rate of disappearance from the luminal perfusate and expressed in μmol/min per g dry weight. D-Fructose was determined using a test kit (Boehringer, UK).

Membrane vesicle preparation

Brush-border and basolateral membrane vesicles were made by a modified version of the simultaneous dual preparation method described by Maenz and Cheeseman [18]. Briefly, two rats were killed by cervical dislocation and mucosal scrapings homogenised in 35 ml ice-cold 20 mM imidazole buffer, pH 7.5 containing 250 mM mannitol and 0.1 mM PMSF (buffered mannitol) using a Kinematica Polytron homogeniser (4 times 30-s bursts using the large probe at setting 7). After filtration through nylon gauze, the homogenate was centrifuged at 2500 g for 15 min using a Sorvall centrifuge. The resulting supernatant was then centrifuged at 21000 g to produce a double layer pellet. The upper, soft layer was resuspended using a 21-gauge needle in buffered mannitol, containing 0.1 mM PMSF and 0.1 mM $MgSO_4$ and made up to 30 ml containing 12% v/v Percoll. The suspension was centrifuged for 60 min at 45000 g using an SW27 rotor in a Beckman L5 preparative ultracentrifuge. The eighth to 15th fractions (1 ml) were pooled, diluted fivefold with buffered mannitol and centrifuged at 45000 g for 1 h to yield the basolateral pellet. The lower, hard layer was resuspended in 22 ml buffered mannitol containing 10 mM $MgCl_2$. After incubation on ice for 20 min with one inversion at 10 min, the solution was centrifuged at 3000 g for 10 min. The supernatant was centrifuged at 27000 g for 30 min to give the final brush-border pellet. Brush-border and basolateral membrane vesicles showed a 20-fold and a 14-fold enrichment in the specific activities of sucrase [19] and vanadate-sensitive Na^+/K^+-ATPase [17] respectively. The purity of the vesicle preparations was also assessed by Western blotting of the marker enzymes using ECL detection: sucrase-isomaltase was probed with mAb DRBB2/158 (1:100 dilution) kindly provided by Professor A. Quaroni (Department of Physiology, Cornell University, [20]), Na^+/K^+-ATPase was probed with anti-rat Na^+/K^+-ATPase α1 fusion protein (Upstate Biotechnology). The integrity of vesicles was confirmed by electron microscopy, using the negative stain technique of Kessler et al. [21].

Photolabelling

The method was adapted from that of Carter-Su et al. [22]. Freshly prepared brush-border membrane vesicles (250 μl) were incubated for 30 min on ice at 1 mg/ml in 5 mM NaH_2PO_4 buffer, pH 7.4 containing 320 mM sucrose, 1 mM EDTA, 500 mM D- or L-glucose, 0.5 μM [^{3}H]cytochalasin B and 2 mM cytochalasin E. The vesicles were irradiated for 10 min using a 250 watt xenon arc lamp focused at 15 cm. After fivefold dilution with ice-cold buffer, the vesicles were collected at 65000 g for 20 min using a Beckman TL100 centrifuge. The pellet was washed twice, solubilised in SDS-PAGE sample buffer and electrophoresed in 7.5% rod gels for 4 h at 3 mA per rod. Gel slices (3 mm) were washed and incubated overnight in 1 ml 50 mM Tris/HCl buffer, pH 7.4 containing 2% SDS: 250 μl of supernatant was mixed with 3 ml Optiphase for scintillation counting.

Western blotting

SDS-PAGE was performed using the Laemelli system. Samples for Western blotting were solubilised for 5 min at 100° C in loading buffer containing 2% SDS and proteins were separated using 5% stacking and 10% resolving gels. Molecular mass (M_r) was determined using a standard calibration kit (prestained marker mixture, Sigma, UK). Proteins were transferred at 4° C to nitrocellulose at 600 mA for 4 h in phosphate buffer (25 mM Na_2HPO_4 + 25 mM NaH_2PO_4, pH 6.5) containing 0.02% SDS. Uniformity of gel loading and protein transfer to the filters were assessed by Ponceau S staining. Immunoblotting was performed using affinity-purified polyclonal antibodies raised in the rabbit to the C-terminal sequences of GLUTs 1–5 [23–25]. Filters were blocked overnight at room temperature in 20 ml PBS (pH 7.4)/EDTA (2 mM)/Triton X-100 (0.2%) buffer, containing 5% non-fat dry milk (Marvel) when probing for GLUTs 2–5, or 3% BSA when probing for GLUT1. They were then incubated for 2 h at room temperature in 4 ml PBS/EDTA/Triton X-100 buffer containing 1% non-fat dry milk with the appropriate antibody and washed three times for 15 min at room temperature in 50 ml PBS/EDTA/Triton X-100 buffer. After incubation of filters for 3 h with 5 ml PBS/EDTA/Triton X-100 buffer containing 1% non-fat dry milk and 1 μCi (or 37 kBq) ^{125}I goat anti-rabbit IgG and washing, immunoreactive bands were visualised using autoradiography with Fuji RX X-ray film and an intensifying screen at −80° C. Autoradiographic bands were quantitated using an LKB Ultroscan XL densitometer; calibration gels with different loadings ensured that experimental band intensities were within the known linear range. In some instances, bands were visualised using an ECL kit (Amersham International), in which case the second antibody was a peroxidase conjugate of goat anti-rabbit IgG.

Fructose transport in membrane vesicles

For D-fructose uptake studies, brush-border or basolateral membrane vesicles were resuspended at 5 mg/ml in 20 mM HEPES

buffer, pH 7.4, containing 250 mM mannitol and 0.1 mM $MgSO_4$ (resuspension buffer). The uptake medium was 2 mM D-fructose and D-[^{14}C]fructose (1.25 μCi or 46.25 kBq per 100 μl) in resuspension buffer containing 2 mM NaN_3. Uptake was initiated by mixing 25 μl of prewarmed vesicle suspension at 25° C with 25 μl prewarmed uptake medium. After incubation for the desired time, uptake was terminated by dilution with 4 ml of ice-cold stop solution (resuspension buffer containing 1 mM $HgCl_2$ and 1 mM NaN_3), followed by a rapid filtration under vacuum through a Millipore filter (type DA, 0.65 μm). The collected membranes were washed with a further 4 ml stop solution and the filter was incubated overnight in 3 ml Optiphase before counting on an LKB 212 Minibeta scintillation counter. Sigrist-Nelson and Hopfer [10] and Crouzoulon and Korieh [11] have each reported that the time-course of fructose uptake by brush-border membrane vesicles from normal rats, which occurs by GLUT5 (see below), is linear for up to 1 min. As in their experiments, we therefore used an incubation time of 45 s. However, with vesicles from diabetic rats, when GLUT2 was also present, the incubation time was reduced to 10 s to ensure that the increased rate of uptake did not compromise kinetic measurements. The uptake data were corrected for non-specific binding to the filter and trapped counts by a zero time point measurement in which uptake medium was mixed with 4 ml stop solution before mixing with vesicles. Uptake rates were corrected for the diffusive component where appropriate by replacing D-fructose with L-glucose. Data were obtained from at least three different vesicle preparations with triplicate measurements for each incubation. The data are presented as pmol per s per mg protein. The uptake of D-fructose by basolateral membrane vesicles was measured in the same way as for brush-border membrane vesicles.

When the extent of inhibition of 1 mM D-fructose uptake by 100 mM D-glucose was determined in brush-border membrane vesicles, the uptake medium was 20 mM HEPES buffer, pH 7.4 containing 50 mM mannitol, 2 mM D-[^{14}C]fructose and 200 mM D-glucose. The uptake of D-glucose alone by brush-border membrane vesicles was determined as for D-fructose, except that the uptake medium was 20 mM HEPES buffer, pH 7.4 containing 50 mM mannitol, 200 mM NaSCN and 0.2 mM D-[^{14}C]glucose and the stop buffer also contained 0.1 mM phlorizin. Under these conditions, the peak to equilibrium ratio for D-glucose uptake was 2.0, confirming the integrity of the vesicles. Protein concentration was determined using the Bio-Rad assay kit.

Chemicals

[4(*n*)-^{3}H]cytochalasin B (17.7 Ci/mmol or 655 GBq/mmol), D-[U-^{14}C]fructose (285 mCi/mmol of 10.5 GBq/mmol) and D-[U-^{14}C]glucose (230 mCi/mmol or 8.51 GBq/mmol) were from Amersham International, UK; L-[1-^{14}C]glucose (55 mCi/mmol or 2.04 GBq/mmol) and [^{125}I]goat anti-rabbit IgG (6.9 μCi/μg or 255 kBq/μg) were from NEN Dupont. All other biochemicals were obtained from Sigma, UK.

Statistical analysis

Values are presented as means ± SEM and were tested for significance using either a paired or unpaired Student's *t*-test as appropriate

Results

Photolabelling of a D-fructose transporter in brush-border membrane vesicles with cytochalasin B

Vesicle uptake experiments have previously indicated that a distinct and specific transport system for D-fructose is present in rat intestinal brush-border [10, 11]. In order to demonstrate the existence of such a facilitative D-fructose transporter in the brush-border membrane of jejunum from normal rats, vesicles were incubated with 0.5 μM [^{3}H]cytochalasin B in the presence 2 mM cytochalasin E to minimise non-specific binding of cytochalasin B and 500 mM L-glucose. Following irradiation and SDS-PAGE, a single band of radioactivity was observed with an apparent M_r of 55 kDa (Fig. 1). Labelling of the cytochalasin-B-binding protein was inhibited by 80% when L-glucose was replaced by D-fructose, demonstrating a stereospecificity consistent with that of a facilitative D-fructose transporter.

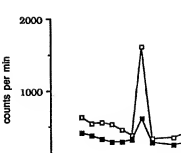

Fig. 1 Electrophoretic profile of brush-border membrane vesicles photolabelled with cytochalasin B. Vesicles prepared from the jejuna of control rats were photolabelled with 0.5 μM [^{3}H]-cytochalasin B by irradiation with a 250 watt xenon arc lamp at 4° C in the presence of 2 mM cytochalasin E and either 500 mM L-glucose (□) or 500 mM D-fructose (■); membrane protein (250 μg) was then analysed on 7.5% SDS-PAGE gels. The molecular mass of the labelled band is 55 kDa. For full details, see Materials and methods

Perfusion studies demonstrating that a phloretin-sensitive D-fructose transporter is detectable in the brush-border membrane of diabetic but not normal rats

Single-pass perfusion experiments in vivo were undertaken to investigate the nature of D-fructose uptake. In these experiments, the jejunal lumen was first perfused for a control period of 0–60 min with 5 mM D-fructose alone and then for an experimental period of 60–120 min with a fresh solution of 5 mM D-fructose containing 0.5 mM phloretin as described in detail in Materials and methods. D-Fructose was taken up by the jejunum in normal rats during the 0–60 min control period at a rate of 1.48 ± 0.08 μmol·min^{-1}·g^{-1} dry wt (Fig. 2a, b): the transporter mediating D-fructose uptake was phloretin insensitive, since uptake during the 60–120 min experimental period was unaffected by phloretin. D-Fructose uptake characteristics were, however, very different when measured in the jejunum of rats with chronic diabetes, 10 days after the injection of streptozotocin. The rate of D-fructose uptake during the 0–60 min control period was

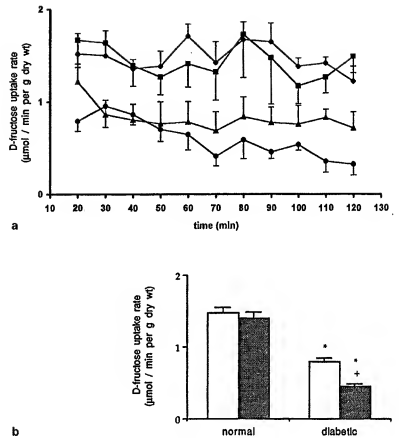

Fig. 2a, b Demonstration by in vivo perfusion of the appearance of a phloretin-sensitive D-fructose transporter in the brush-border membrane of jejunum in 10-day streptozotocin-diabetic rats. Jejunal loops were perfused in vivo in single-pass mode with 5 mM D-fructose for a control period from 0–60 min without phloretin and then for an experimental period from 60 to 120 min, either without phloretin to test the viability of the preparation, or with 0.5 mM phloretin. a Time-courses are presented for jejunum from normal rats perfused without phloretin (■, n=4) and with phloretin in the experimental period (◆, n=4) and also for jejunum from diabetic rats perfused without phloretin (▲, n=6) and with phloretin in the experimental period (●, n=6). For full details, see Materials and methods section. b The average rates of D-fructose uptake over control (*open bar*) and experimental (*hatched bar*) periods are presented for the perfusions of jejunum from normal and diabetic rats in which phloretin was present during the experimental period. Values are given as mean ± SEM. * P<0.001 by unpaired t-test for comparison of the corresponding perfusion periods between normal and diabetic rats, + P<0.001 by paired t-test for comparison of the control and experimental perfusion periods for diabetic rats

54% of that in normal rats (P<0.001). Moreover, D-fructose uptake could be inhibited by phloretin during the 60–120 min experimental period to a final level that was 56% of that in the control perfusion period for diabetic rats (P<0.001). Additional experiments, in which phloretin was omitted from the second perfusion period, showed no significant difference in rates between the control and experimental periods, confirming that the preparation was viable throughout the full length of the perfusion for both normal and diabetic rats: the time-courses for these additional controls are shown in Fig. 2a, but the corresponding data have been omitted from the histogram in Fig. 2b which shows the average rates of D-fructose uptake in normal and diabetic rats in vivo only for those perfusions in which phloretin was present during the experimental period.

Plasma D-glucose levels are very high in diabetic rats, more than 25 mM in the present study. It was therefore important to use an alternative perfusion technique to eliminate any possible metabolic or transport effects of D-glucose that could have affected D-fructose uptake in diabetic rats compared with normal rats in vivo. For this purpose, we used the isolated, recirculated loop in vitro perfused with 5 mM fructose in the absence (0–50 min control period) and presence of 0.5 mM phloretin (50–100 min experimental period): no D-glucose was present. In this case, the upake rate for diabetic rats during the control period was enhanced by 49% (P<0.05) compared with that for normal rats (Fig. 3), in contrast to the diminution seen with in vivo perfusions (Fig. 2b). However, as was the case with in vivo perfusions, phloretin had no effect on D-fructose uptake by the jejunum from normal rats, whereas in diabetic rats it significantly diminished D-fructose uptake to 59% (P<0.02) of the rate in the absence of phloretin (Fig. 3). Both sets of perfusion experiments are therefore consistent with the idea that adaptation to diabetes results in the appearance at the brush-border of a transporter that is sensitive to phloretin and so is distinct from the transporter responsible for uptake of D-fructose in normal rats.

Western blot studies of the adaptation of the membrane distribution and levels of D-fructose transporters to diabetes

The facilitative transporter isoforms involved in D-fructose transport were analysed by Western blotting of both

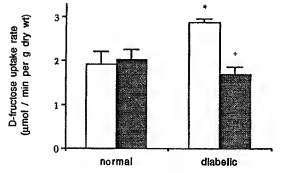

Fig. 3 Demonstration by in vitro perfusion of the appearance of a phloretin-sensitive D-fructose transporter in the brush-border membrane of jejunum in 10-day streptozotocin diabetic rats. Jejunal loops were perfused in vitro in recirculated mode with 5 mM D-fructose for a control period from 0 to 50 min without phloretin and then for an experimental period from 50 to 100 min in the presence of 0.5 mM phloretin. Additional perfusions in which phloretin was not present during the experimental period showed that preparations from normal and diabetic rats were viable for the full length of the perfusion (data not shown). For full details, see Materials and methods. The average rates of D-fructose uptake over control (*open bar*) and experimental (*hatched bar*) periods are presented for the perfusions of jejunum from normal and diabetic rats in which phloretin was present during the experimental period. Values are given as mean ± SEM (n=5). * P<0.05 by unpaired t-test for comparison of the 0–50 min control perfusion period between normal and diabetic rats; + P<0.02 by paired t-test for comparison of the control and experimental perfusion periods for diabetic rats

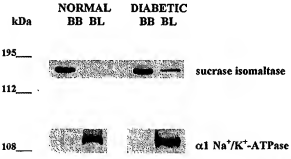

Fig. 4 Characterisation of rat intestinal brush-border and basolateral membrane vesicle preparations from normal and diabetic rats. Brush-border (*BB*) and basolateral (*BL*) membrane vesicles were prepared from jejuna of normal or diabetic rats 10 days after streptozotocin injection. Vesicle protein (40 μg) was separated on 10 or 7.5% SDS-PAGE gels and transblotted onto nitrocellulose membranes. Membranes were probed with either a monoclonal antibody to sucrase-isomaltase or a polyclonal antibody to α1 Na^+/K^+-ATPase and binding was detected by ECL (see Materials and methods)

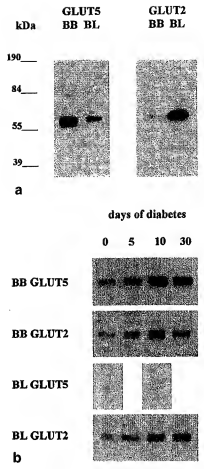

Fig. 5a, b Adaptation of GLUT5 and GLUT2 in rat intestinal brush-border and basolateral membranes to diabetes. Brush-border (*BB*) and basolateral (*BL*) membrane vesicles were prepared from the jejunal mucosa of normal rats (0 days) or diabetic rats 5, 10 and 30 days after the injection of streptozotocin. Vesicle protein (40 μg) was separated on 10% SDS-PAGE gels and transblotted onto nitrocellulose membranes. Membranes were probed with antibodies to GLUT5 and GLUT2 and antibody binding detected by incubation with [^{125}I]goat anti-rabbit IgG followed by autoradiography. a Distribution of GLUT5 and GLUT2 in vesicles from normal (0 days) rats. b The adaptation of GLUT5 and GLUT2 to diabetes. Photographic exposures are constant within any one group from 0 to 30 days, but differ between groups. GLUT5 in BL membrane vesicles detected by ECL using samples at 0 and 10 days of diabetes; although a comparison sample from BB vesicles was strongly positive (not shown), GLUT5 was not detected in BL vesicles in this instance

brush-border and basolateral membrane vesicles using specific antibodies directed against the C-termini of GLUTs1–5. The interpretation of these experiments and the transport measurements that follow depend crucially on demonstrating that any cross-contamination between membranes which could occur during preparation is very low for normal rats and, moreover, remains low for diabetic rats. That this is indeed so is shown in Fig. 4; in both cases sucrase-isomaltase and Na^+/K^+-ATPase are present almost exclusively in brush-border and basolateral membrane vesicles respectively as determined by Western blotting. GLUT3 and GLUT4 could not be de-

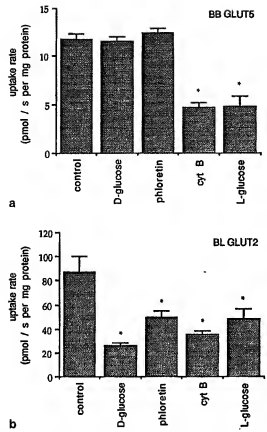

Fig. 6 D-Fructose transport characteristics of a brush-border (*BB*, GLUT5) and b basolateral (*BL*, GLUT2) membrane vesicles prepared from the jejuna of normal rats. The rate of uptake of 1 mM D-fructose was determined either alone (control) or in the presence of either 100 mM D-glucose, 0.1 mM phloretin or 20 μM cytochalasin B and compared with the rate of uptake of 1 mM L-glucose alone. Values are given as mean ± SEM; * $P<0.001$ for comparison with normal values

tected in either brush-border or basolateral membrane vesicles, when compared against positive standards of membranes from brain and adipose tissue respectively. Furthermore, only very low levels of GLUT1 were detected in both brush-border and basolateral membrane vesicles, when compared against a positive standard of red cell ghosts; the levels did not change during diabetes (data not shown). Miyamoto et al. [7] have previously reported that no transcripts of GLUTs 1, 3 and 4 could be detected in intestinal mucosa. Subsequent experiments therefore concentrated on GLUT2 and GLUT5, which were the major transporter isoforms detected.

With antibody to GLUT5, the only band detected in brush-border membrane vesicles from the jejunum of normal rats was a strong band with an apparent M_r of 58 kDa, whereas only a faint band was detected in basolateral membrane vesicles (Fig. 5a); in some instances, no GLUT5 was detected in preparations of basolateral

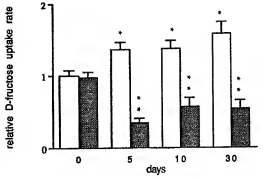

Fig. 7 Adaptation of D-fructose transport in brush-border membrane vesicles to streptozotocin-induced diabetes. Brush-border membrane vesicles were prepared from jejuna of normal rats (0 days) or rats 5, 10 and 30 days after the injection of streptozotocin. The rate of uptake of 1 mM D-fructose was then determined in the absence (*open bars*) or presence of 100 mM D-glucose (*hatched bars*). Rates were corrected for the diffusive component measured with 1 mM L-glucose and expressed relative to that at 0 days in the absence of D-glucose. Values are given as mean ± SEM; * $P<0.05$ and ** $P<0.01$ for comparison with corresponding normal (0 day) values

membrane vesicles (Fig. 5b). In contrast, with antibody to GLUT2, a single, strong band with an apparent M_r of 62 kDa was detected in basolateral membrane vesicles, whereas only a very faint band was detected in brush-border membrane vesicles (Fig. 5a). The induction of experimental diabetes by the injection of streptozotocin resulted in significant increases in the band intensities of both GLUT5 and GLUT2 in brush-border membrane vesicles and also of GLUT2 in basolateral membrane vesicles. The very faint band for GLUT5 in basolateral membrane vesicles was either unchanged by diabetes or GLUT5 remained undetectable (Fig. 5b). Maximal enhancement of signal intensities was observed 10 days after streptozotocin, namely 2.8-fold and 6.5-fold for GLUT5 and GLUT2 respectively in brush-border membrane vesicles and 1.8-fold for GLUT2 in basolateral membrane vesicles.

The contribution of GLUT5 and GLUT2 to D-fructose transport by brush-border membrane vesicles from diabetic rats

In order to study the adaptation of brush-border membrane transport of D-fructose to diabetes, it was necessary to identify differences in transport characteristics between GLUT5 and GLUT2. Comparison of brush-border and basolateral membrane preparations from normal rats, whose major D-fructose transporters are GLUT5 and GLUT2 respectively (Fig. 5a), demonstrated that GLUT5 and GLUT2 have very different transport properties. Both transporters were strongly inhibited by preincubation of vesicles for 15 min with 20 μM cytochalasin B, which diminished the rate of uptake of 1 mM D-fructose to the

same as that for the diffusive component measured by 1 mM L-glucose (Fig. 6a, b). However, whereas 1 mM D-fructose uptake by GLUT5 was unaffected by either by 0.1 mM phloretin or 100 mM D-glucose, uptake by GLUT2 was strongly inhibited by either phloretin or D-glucose, so that uptake rates were similar to those in the presence of cytochalasin B or for L-glucose.

The equilibrium value of D-fructose transport in brush-border membrane vesicles from normal rats was 1552 ± 151 pmol/mg protein, which is within the range of values from approximately 1400 to 1800 pmol/mg protein previously reported [10, 11]. The value of 1390 ± 79 pmol/mg protein for 10-day streptozotocin-diabetic rats was not significantly different, indicating that no significant changes in vesicularity were induced by diabetes, as noted by other workers for 10- to 35-day diabetic rats [26, 27]. In broad agreement with this view, the rate of transport of L-glucose at 5 and 10 days of diabetes was not significantly different from that for normal rats (Fig. 6a); however, L-glucose transport was enhanced 126% ($P<0.05$) at 30 days of diabetes, indicating some increase in permeability. When L-glucose transport values were used to correct D-fructose transport for the diffusive component, it was apparent that the facilitated component of 1 mM D-fructose transport by brush-border membrane vesicles was enhanced in diabetes (Fig. 7). The maximal enhancement measured was 57% ($P<0.001$) at 30 days, but was not significantly different from that at 10 days.

Since both GLUT5 and GLUT2 were present in the vesicles, it was of interest to resolve their separate contributions to D-fructose transport. As noted, D-glucose inhibits D-fructose uptake by GLUT2 but not GLUT5. With vesicles from normal rats, then, 100 mM D-glucose had no effect on the rate of uptake of D-fructose: however, it significantly inhibited 1 mM D-fructose uptake at 5, 10 and 30 days of diabetes. Thus diabetes resulted in the appearance of a contribution to D-fructose transport by GLUT2 in brush-border membrane vesicles. The residual contribution of the corrected transport rates in the presence of D-glucose represented that occurring by GLUT5. Of note is the fact that the GLUT5 contribution to D-fructose transport in diabetes was significantly below that of the control rate (Fig. 7), despite the fact that the concentration of GLUT5 had significantly increased (Fig. 5b); the comparison between transport and Western blotting data can be made directly, since, as shown, there were no significant changes in vesicularity induced by diabetes. Thus the intrinsic activity of GLUT5 at 1 mM D-fructose was diminished in diabetes. In order to determine whether K_t (the transport constant) or J_{max} (the maximal intrinsic activity) was altered in diabetes, it would have been necessary to study D-fructose transport over a wide range of concentrations; however, this was not attempted, since it was not feasible to maintain the appropriate ratios of inhibitors to D-fructose. It was not possible to make statements about possible changes in the intrinsic activity of GLUT2 in brush-border membranes, because its transport contribution in normal rats was not detectable.

Discussion

In vivo perfusions revealed that D-fructose uptake was significantly diminished in diabetic compared with normal rats (Fig. 2). Both metabolic and transport effects are likely to contribute to the difference in behaviour. Thus Holloway and Parsons [28] have reported that D-glucose inhibits the uptake of D-fructose in perfused intestine through an effect on metabolism. Since the concentration of D-glucose in mucosa is determined broadly by its plasma concentration [29], D-fructose uptake in vivo will be inhibited in diabetic compared with normal rats. Cheeseman [14] has reported that the major basolateral transporter of D-fructose in rat jejunum is GLUT2, wich also transports D-glucose and, indeed, can take up D-glucose into the mucosa from the blood under appropriate circumstances. In diabetic rats, therefore, D-glucose can compete with D-fructose for the GLUT2 transporter more effectively than in normal rats and inhibit exit. The in vitro perfusions were designed to avoid these potential complications by having no D-glucose present. The consequence was that D-fructose uptake was actually enhanced with jejunum from diabetic rats compared with normal rats (Fig. 3).

Csaky and Fisher [30] have reported that luminal phloretin does not inhibit D-fructose uptake by everted sleeves of jejunum from normal rats. Our observation that luminal phloretin had no effect on D-fructose uptake in both in vivo and in vitro perfusion experiments with jejunum from normal rats (Figs. 2, 3) therefore confirmed their findings, showing the brush-border D-fructose transporter in normal rat jejunum to be phloretin insensitive. In contrast, our perfusion experiments revealed that luminal phloretin significantly inhibited D-fructose uptake in 10-day streptozotocin-diabetic rats. Czaky and Fisher [30] reported that in fact it is possible for phloretin to inhibit D-fructose uptake when present at the serosal side of normal rat jejunum, showing that the basolateral D-fructose transporter is phloretin-sensitive. However, the possibility that luminal phloretin in our perfusions with diabetic rats might have gained access to the serosal side and prevented D-fructose exit seems very unlikely, since it had no effect in normal rats. The perfusion experiments therefore demonstrate that adaptation to diabetes results in the appearance in the brush-border of a D-fructose transporter which is phloretin-sensitive and which is therefore distinct from the transporter present in normal rats. When taken in conjunction with Csaky and Fisher's work [30], perfusion studies alone suggest that the new transporter appearing in the brush-border of diabetic rats might well be the transporter that normally is exclusively basolateral.

Western blotting revealed that the major facilitative transporters in normal rats are GLUT5 at the brush-border and GLUT2 at the basolateral membrane: trace amounts only of GLUT5 and GLUT2 were detected in brush-border and basolateral vesicles respectively (Fig. 5a). Although, as shown in Fig. 4, the preparations were highly purified, a very small amount of cross-contamina-

tion is unavoidable. Although it cannot be stated definitively, such cross-contamination might therefore account for the trace amounts of GLUT5 and GLUT2 found in vesicles from basolateral and brush-border membranes of normal rats respectively. Diabetes caused a marked increase in GLUT5 and GLUT2 protein in brush-border membrane vesicles, reaching maximum values of 2.8- and 6.5-fold respectively after 10 days (Fig. 5). GLUT2 levels in the basolateral membrane vesicles also increased 1.8-fold, but GLUT5 was not detected. Chowrimootoo et al. [31] have previously reported the presence of GLUT2 immuno-like reactivity in brush-border membrane vesicles from diabetic rats. Miyamoto et al. [6] have reported a threefold increase in GLUT2 levels in basolateral membrane vesicles after 10 days of diabetes. Since the degree of purity of vesicle preparations from normal and diabetic rats was similar (Fig. 4), the difference in relative enhancements of GLUT2 levels in brush-border and basolateral membrane vesicles is clearly consistent with the idea that GLUT2 is being expressed at the brush-border membrane in diabetes.

Since both GLUT5 and GLUT2 were present in brush-border membrane vesicles from diabetic rats, it became necessary to distinguish between their transport properties in order to determine their different contributions to the adaptation of D-fructose transport. In brush-border membrane vesicles from normal rats, where the major D-fructose transporter is GLUT5, cytochalasin B inhibited 1 mM D-fructose transport to the level for the diffusive component determined with 1 mM L-glucose (Fig. 6a) this was consistent with the ability of D-fructose to inhibit [^{3}H]cytochalasin B photo-labelling by as much as 80% (Fig. 1). In contrast, a 1:10 ratio of phloretin to D-fructose had no effect on the transport of 1 mM D-fructose in brush-border membrane vesicles from normal rats, an observation which is in good in agreement with the perfusion studies in whole jejunum (Figs. 2, 3) and studies with everted sleeves [30]. Similarly, a 100-fold ratio of D-glucose to D-fructose had no effect on D-fructose transport in brush-border vesicles from normal rats. The latter observation is in very good agreement with those of Sigrist-Nelson and Hopfer [10] and of Crouzoulon and Korieh [11], who found that ratios of 100 and 50 respectively of D-glucose to D-fructose had no effect on the transport of 1 mM D-fructose in brush-border membrane vesicles. The rat brush-border D-fructose transport system (GLUT5) in normal rats therefore displays a very strict stereospecificity. This contrasts with the properties of rat GLUT5 expressed in oocytes observed by Rand et al. [32], who found that transport of 1 mM D-fructose was inhibited by 50 mM D-glucose and that GLUT5 transported deoxyglucose. Moreover, cytochalasin B did not inhibit D-fructose transport by GLUT5 in oocytes, whereas it labelled GLUT5 (Fig. 1) and inhibited GLUT5 in brush-border membrane vesicles (Fig. 6). It is therefore clear that the stereospecificity of rat GLUT5 is altered when expressed in oocytes: similar changes in stereospecificity have also been reported for rabbit GLUT5 when expressed in oocytes [33].

In basolateral membrane vesicles from normal rats, where GLUT2 is the major D-fructose transporter, the transport of 1 mM D-fructose was strongly inhibited by 20 μM cytochalasin B, a 1:10 ratio of phloretin to D-fructose and a 100-fold ratio of D-glucose to D-fructose; the residual transport activity was the same as observed for the diffusive component with 1 mM L-glucose (Fig. 6b). These findings are in full agreement with those of Cheeseman [14], who has demonstrated that GLUT2 is the D-fructose transporter of the basolateral membrane in rat, and fit well with those of Csaky and Fisher, who reported the sensitivity of basolateral D-fructose uptake to phloretin [30]. When taken with the Western blotting data, the findings show that the phloretin-sensitive transporter detected by perfusion studies in the brush-border of intact jejunum in diabetic rats is GLUT2.

When GLUT5 and GLUT2 were present in brush-border membrane vesicles from diabetic rats, we used 100 mM D-glucose to inhibit the GLUT2 contribution to the transport of 1 mM D-fructose and attributed the residual contribution to GLUT5 (after rates had been corrected for the diffusive component). At 10 days of diabetes, the residual GLUT5 contribution was 56% of the normal (0 day) GLUT5 value (Fig. 7). Since Western blot data showed that the GLUT5 level at 10 days of diabetes was 2.8-fold greater than normal, it was apparent that the intrinsic activity of GLUT5 was about one-fifth of its normal value (at 1 mM D-fructose). The conclusion is supported by the perfusion experiments with isolated loops in vitro, which are free of the significant metabolic/transport effects of elevated blood glucose that apply with in vivo perfusions. In order to circumvent similar effects occurring in isolated loops, it was necessary to use phloretin instead of D-glucose in the lumen to inhibit GLUT2 (Figs. 3, 6). At 5 mM D-fructose, the residual uptake rate attributable to GLUT5 for 10-day diabetic rats measured in the presence of phloretin was 88% of the normal GLUT5 rate in the absence of phloretin. The difference was not in fact significant, but, as noted, GLUT5 levels were enhanced 2.8-fold. Rates of uptake in perfusion experiments often reflect a combination of luminal, metabolic and serosal steps. When isolated loops were perfused with 5 mM D-fructose in the absence of D-glucose, no D-fructose appeared in the serosal medium. The uptake rate therefore most probably reflected the rate of brush-border transport, for not only was the serosal side not involved, but metabolism was not saturated and therefore unlikely to be rate-limiting. The findings indicate then that the intrinsic activity of GLUT5 is diminished in diabetes to about one-third of its normal value (at 5 mM D-fructose).

The adaptive responses described are likely to have significant functional consequences. Studies with vesicles and with perfused isolated loops in the absence of D-glucose suggested that overall uptake of D-fructose uptake is enhanced in diabetes. However, these conditions did not reflect the true in vivo situation, for the perfusion of jejunal loops in vivo revealed that diabetes actually results in a diminution in overall D-fructose uptake. The contributing factors include a significant, inhibitory metabolic ef-

fect caused by the presence in mucosa of D-glucose at concentrations comparable to those in blood [28, 29] and a diminished intrinsic activity for GLUT5. Although GLUT2 is expressed in the brush-border of diabetic rats, the additional transport component attributable to GLUT2 in isolated loops in vitro (phloretin-inhibitable, Fig. 3) or vesicles (D-glucose-inhibitable, Fig. 7), and potentially available in vivo, is only partially realised (Fig. 2). Clearly, the inhibitory metabolic effect will apply equally to D-fructose whether it is transported by GLUT2 or GLUT5. However, a further consideration is that GLUT2 transports D-glucose, so that the high mucosal D-glucose concentration in diabetic rats diminishes D-fructose exit. That D-fructose can be transported by GLUT2 across the brush-border membrane of diabetic rats is clearly shown by the fact that phloretin inhibits D-fructose uptake in vivo.

In conclusion, we have shown that the major D-fructose transporters of brush-border and basolateral membranes in normal rats maintained on a standard chow diet, GLUT5 and GLUT2 respectively, exhibit marked differences in stereospecificity: GLUT5 is not inhibited by D-glucose or phloretin, whereas GLUT2 is strongly inhibited by both. Adaptation to diabetes is accompanied by significant enhancement in the levels of GLUT5 and GLUT2 in brush-border and basolateral membranes respectively. Moreover, GLUT2 is also expressed in the brush-border membrane of diabetic rats, its level increasing as diabetes progresses. The contribution of GLUT2 to brush-border D-fructose transport is readily detectable either in perfusion studies of whole intestine from diabetic rats as a phloretin-sensitive component that is not detectable in normal rats, or in brush-border membrane vesicles from diabetic rats as a D-glucose-inhibitable component that is not detectable in vesicles from normal rats. In addition, the intrinsic activity of GLUT5 is diminished in diabetes. Although D-fructose transport is enhanced in isolated loops in vitro and brush-border membrane vesicles, the inhibitory effect of high mucosal D-glucose concentrations on D-fructose metabolism and the ability of GLUT2 to transport both D-glucose and D-fructose contribute to the fact that inhibition of D-fructose uptake is observed in diabetic rats in vivo.

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